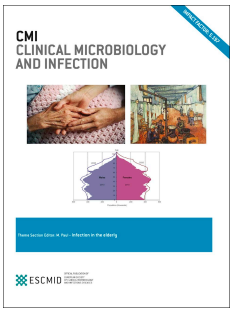
Journal Pre-proof



Pulmonary long-term consequences of COVID-19 infections after hospital discharge

J.R. Blanco, M.J. Cobos-Ceballos, F. Navarro, I. Sanjoaquin, F. Arnaiz de las Revillas, E. Bernal, L. Buzon-Martin, M. Viribay, L. Romero, S. Espejo-Pérez, B. Valencia, D. Ibañez, D. Ferrer-Pargada, D. Malia, F.G. Gutierrez-Herrero, J. Olalla, B. Jurado-Gamez, J. Ugedo

PII: S1198-743X(21)00101-4

DOI: https://doi.org/10.1016/j.cmi.2021.02.019

Reference: CMI 2434

To appear in: *Clinical Microbiology and Infection*

Received Date: 11 November 2020

Revised Date: 9 February 2021

Accepted Date: 18 February 2021

Please cite this article as: Blanco J, Cobos-Ceballos M, Navarro F, Sanjoaquin I, Arnaiz de las Revillas F, Bernal E, Buzon-Martin L, Viribay M, Romero L, Espejo-Pérez S, Valencia B, Ibañez D, Ferrer-Pargada D, Malia D, Gutierrez-Herrero F, Olalla J, Jurado-Gamez B, Ugedo J, Pulmonary long-term consequences of COVID-19 infections after hospital discharge, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2021.02.019.

Title: Pulmonary long-term consequences of COVID-19 infections after hospital discharge

AUTHORS:

JR. Blanco [1,2]

MJ. Cobos-Ceballos [3,4]

F. Navarro [5]

I. Sanjoaquin [6]

F. Arnaiz de las Revillas [7]

E. Bernal [8]

L. Buzon-Martin [9]

M. Viribay [10]

L. Romero[2]

S. Espejo-Pérez [3,11]

B. Valencia [12]

D. Ibañez [13]

D. Ferrer-Pargada [14]

D. Malia [15]

FG. Gutierrez-Herrero [16]

J. Olalla [5]

B. Jurado-Gamez [3,4]

J. Ugedo [17]

1. Servicio de Enfermedades Infecciosas. Hospital Universitario San Pedro, Logroño, La Rioja, SPAIN.
2. Centro de Investigación Biomédica de La Rioja, Logroño, La Rioja, SPAIN
3. Instituto Maimónides de Investigación Biomédica de Córdoba. Universidad de Córdoba. SPAIN
4. Servicio de Neumología. Hospital Universitario Reina Sofía, Córdoba. SPAIN
5. Servicio de Medicina Interna. Hospital Costal de Sol, Marbella. Málaga. SPAIN
6. Servicio de Enfermedades Infecciosas. HCU Lozano Blesa, Zaragoza. SPAIN
7. Servicio de Enfermedades Infecciosas. H Universitario Marqués de Valdecilla, Santander. SPAIN
8. Sección de Enfermedades Infecciosas. Hospital General Universitario Reina Sofía de Murcia. Universidad de Murcia. SPAIN
9. Servicio de Medicina Interna. Hospital Universitario de Burgos. SPAIN
10. Vitro, Labora
11. Servicio de Radiología. Hospital Universitario Reina Sofía, Córdoba. SPAIN
12. Servicio de Neumología. Hospital Costal de Sol, Marbella. Málaga. SPAIN
13. Servicio de Radiología. HCU Lozano Blesa, Zaragoza. SPAIN
14. Servicio de Neumología. H Universitario Marqués de Valdecilla, Santander. SPAIN
15. Servicio de Neumología. Hospital General Universitario Reina Sofía de Murcia. SPAIN
16. Servicio de Neumología. Hospital Universitario de Burgos. SPAIN
17. Servicio de Neumología. Hospital Universitario San Pedro, Logroño, La Rioja. SPAIN

Keywords: COVID-19, Intracellular adhesion molecule, Lung diffusion capacity, Osteoprotegerin, Plasminogen activator inhibitor, Tissue inhibitor of matrix metalloproteinases, Tomography

Corresponding author:
Dr. José-Ramón Blanco
Hospital San Pedro – Centro de Investigación Biomédica de La Rioja (CIBIR)
Departamento de Enfermedades Infecciosas
Piqueras 98, 26006 Logroño, La Rioja, Spain
Telephone: +34 941298993
Email: jrblanco@riojasalud.es ; jrblancoramos@gmail.com

ABSTRACT

Objectives: COVID-19 survivors are reporting residual abnormalities after discharge from the hospital. Limited information is available about this stage of recovery or the lingering effects of the virus on pulmonary function and inflammation. The aim of this study was to describe lung function and to identify biomarkers in serum and induced sputum samples from patients recovering from COVID-19 hospitalisation.

Methods: Patients admitted to Spanish hospitals with laboratory-confirmed COVID-19 infection by a real-time PCR (RT-PCR) assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were recruited for this study. Each hospital screened their lists of discharged patients at least 45 days after symptom onset. SARS-CoV-2-infected patients were divided into mild/moderate and severe disease groups according to the severity of their symptoms during hospitalisation. Patients' epidemiological and medical histories, comorbidities, chronic treatments, and laboratory parameters were evaluated. Pulmonary function tests, the standardised 6-minute walk test (6 MWT) and chest computed tomography (CT) were also performed. The levels of proteases, their inhibitors, and shed receptors were measured in serum and induced sputum samples.

Results: A total of 100 patients with respiratory function tests were included in this study. The median number of days after the onset of symptoms was 104 (IQR 89.25, 126.75). COVID-19 was severe in 47% (47/100) of patients. CT was normal in 48% (48/100) of patients. Lung function was normal (FEV1 ≥80%, FVC ≥80%, FEV1/FVC ≥0.7, and diffusing capacity for carbon monoxide [DLCO] ≥80%) in 92% (92/100), 94% (94/100), 100% (100/100) and 48% (48/100) of patients, respectively. Multivariate analysis showed that a DLCO <80% (OR 5.92; 95%CI 2.28-15.37; p <0.0001) and a lower serum LDH level (OR 0.98; 95%CI 0.97-0.99) were associated with the severe disease group of SARS-CoV-2 during hospital stay.

Conclusions: A diffusion deficit (DLCO <80%) was still present after hospital discharge and was associated with the most severe SARS-CoV-2 cases.

INTRODUCTION

Approximately 104 million individuals worldwide have recovered from COVID-19 (https://coronavirus.jhu.edu/map.html). However, some survivors report persistent severe symptoms and organ dysfunction [1]. These symptoms might be, in part, a consequence of the cytokine storm suffered in the acute phase of the infection [2]. Previous studies have shown that higher levels of proinflammatory cytokine responses during the acute phase of other coronavirus infections such as severe acute respiratory syndrome (SARS) [3] and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) [4], were associated with severe lung disease. Unlike previous coronaviruses, COVID-19 does not seem to be just a respiratory affliction; rather, it is a viral infectious process involving multiple systems [5].

Residual lung abnormalities have been found in patients with SARS-CoV-2 1-3 months after discharge from the hospital [6-9]. However, limited information is available about the serum inflammatory state during recovery from SARS-CoV-2. The impact of residual inflammation on the lungs is even rarer. Because the persistence of this inflammatory state in blood and sputum could have important prognostic implications, we performed this study.

METHODS

Participants

This was a prospective study of patients older than 18 years of age who were admitted to different Spanish hospitals with laboratory-confirmed COVID-19 infection by real-time PCR (RT-PCR) assay for SARS-CoV-2. Each hospital screened their lists of discharged patients. These patients were interviewed by phone at least 45 days after symptom onset and asked to collaborate if they met inclusion criteria. Exclusion criteria included patients with a need for prior invasive mechanical ventilation, chronic infectious diseases, chronic lung diseases, concurrent autoimmune or cancer diseases, chronic use of corticosteroids or immunosuppressive therapy, pregnancy, alcohol/drug abuse, or patients whose conditions did not allow participation in this study. The study was approved by the Institutional Research Ethics Committees. All participants provided written informed consent.

Patients were divided into mild (mild and moderate) and severe groups according to the severity of their symptoms during their hospital stays. The mild group did not have pneumonia imaging; the moderate group showed pneumonia; and the severe group had dyspnoea, respiratory frequency ≥ 30/minute, blood oxygen saturation ≤93%, PaO_2/FiO_2 ratio <300, and/or lung infiltrates >50% of the lung field within 24-48 hours [10]. Patients requiring invasive mechanical ventilation were excluded because of its impact on systemic inflammation [11].

Epidemiological, medical history, comorbidities, chronic treatments, and laboratory parameters were evaluated. Smoking status was determined from self-administered survey responses. Anthropometric measurements included body mass index (BMI). At least 45 days after symptom onset, pulmonary function testing, standardised 6-minute walk tests (6 MWT) [12], and chest computed tomography (CT) were performed. Lung function included forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio and diffusion capacity of the lung for carbon monoxide (DLCO). Diffusion deficit was considered a DLCO <80% of the predicted value [13]. The 6 MWT, a practical and simple test that provides a global measure of functional capacity, was performed in accordance with international recommendations [12]. Peripheral oxygen saturation (SpO_2) was monitored using a handheld oximeter. ΔSpO_2-6 MWT was defined as the difference between the resting and nadir SpO_2. The 6 MWT distance was also evaluated [14]. CT was considered normal in the absence of ground-glass opacification, crazy-paving patterns, consolidation, or linear opacities [15].

The levels of biomarkers were measured in serum and induced sputum samples. Serum samples were obtained from blood drawn at a date close to the tests already described and stored at -80ºC. Sputum was induced as previously described [16] and stored at -80°C. It was obtained, whenever possible, on the same day as the respiratory function tests. The concentrations of multiple proteases and their inhibitors (plasminogen activator inhibitor [PAI]-1, PAI-2, and tissue inhibitor of matrix metalloproteinases [TIMP]-1). Shed receptor (intracellular adhesion molecule [ICAM]-1, ICAM-3, osteoprotegerin [OPG])

were evaluated in serum and sputum samples. These parameters were analysed in duplicate employing commercially available ELISA kits. The lower detection limits are shown (Supplementary Table 1). All samples were tested individually (one sample per well), but samples from all groups were measured on the same plate. The hook effect, a state of antigen excess relative to the antibody probes, resulting in falsely lowered values, was ruled out after analysing undiluted and diluted samples.

Data analysis

Categorical variables were reported as frequencies and proportions. Continuous variables with a normal distribution are presented as the mean (standard deviation [SD]), and those with a non-normal distribution are presented as the median (interquartile range values [IQR] p25, p75). To compare the demographic and clinical variables between groups, the chi-square test or Fisher's exact test was used for each categorical variable, as appropriate. For quantitative variables, the nonparametric Mann-Whitney U test was used.

Multivariate analysis was carried out using binary logistic regression with the forward conditional method, introducing DLCO (<80 vs. ≥80%) as the dependent variable. Independent variables were all variables that were statistically significant in the bivariate analysis, or clinical implications. The results of the multivariate model were adjusted, and we present the odds ratio and its 95% confidence interval (CI). Statistical significance was set at $p < 0.05$. Analyses were performed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 108 patients were included in this study. Of the sample, 100 had adequate respiratory function tests. Most (69%9 were >50 years (69/100), 64% were male (64/100), and 90% were Caucasian (90/100). The median number of days after the onset of symptoms was 104 (IQR 89.25, 126.75). Common comorbidities included hypertension (25%; 25/100), diabetes mellitus (10%; 10/100), cardiovascular disease (4%; 4/100), and chronic kidney disease (2%; 2/100). Obesity (BMI ≥30%) was present in 37% (37/100), and 59% never had smoked (59/100). Chronic therapy included angiotensin-converting enzyme

inhibitors/angiotensin II receptor blockers use (17%, 17/100), statin use (12%, 12/100), and aspirin use (3%, 3/100). COVID-19 was severe in 47% of patients (47/100). Lung function was normal (FVC ≥80%, FEV1 ≥80%, FVC/FEV1 ≥0.7, and DLCO ≥80%) in 92% (92/100), 94% (94/100), 100% (100/100), and 48% (48/100), respectively. Control CT was normal in 48% (48/100).

Given the high percentage of subjects with DLCO <80% and its involvement in lung damage, this lung parameter was evaluated. Table 1 shows the patient characteristics according to DLCO severity. With the exception of significant data referring to the severity of COVID-19 disease during hospitalisation and the length of hospital, no other significant differences were observed.

Table 2 shows the analytical parameters according to DLCO severity. Finally, Table 3 provides information about the tests carried out and the minimum time elapsed until tests were performed. No differences were observed after analysing ΔSpO_2-6 MWT (data not shown).

Multivariate analysis showed that a DLCO <80% was associated with severe disease in the SARS-CoV-2 group during their hospital stays (OR 5.92; 95% CI 2.28-15.37; p <0.0001) as were lower serum LDH levels (OR 0.98; 95% CI 0.97-0.99; p 0.002).

DISCUSSION

Since the SARS-CoV-2 outbreak, there has been increasing concern about the potential risk of parenchymal fibrosis and lung function impairment. The most important factor is lung diffusion capacity [17]. Zhao et al. [6] reported that three months after COVID-19 discharge, a high percentage of CT abnormalities (70.9%) and DLCO anomalies (16.4%) were still present in recovering patients. Other authors, such as Mo et al. [7], reported that nearly a month after hospital discharge that, regardless of the degree of SARS-CoV-2 severity, no significant differences in FEV1, FVC, or its ratio were observed. However, the DLCO value was significantly lower as the severity of the clinical picture increased (47.2% in total; 30.4% in mild illness and 84.2% in severe pneumoniae). In this study, the authors included a small number of patients with previous pulmonary pathology,

one of the exclusion criteria of our study. Likewise, Frija-Masson et al. [9] also observed that more than half of patients with COVID-19 pneumonia, some of whom had respiratory comorbidities, exhibited abnormal lung function one month after symptom onset, without a clear relationship with pneumonia extent on chest CT. Huang et al. [18] observed that 30 days after discharge from the hospital, patients exhibited nearly significant differences in DLCO values (<80%), 42.5% in non-severe cases, and 75.6% in severe cases ($p < 0.053$). In that study, patients with a previous history of pulmonary resection, neurological disease, or mental illness were excluded. In our study, DLCO findings were close to those observed by Mo et al. [7] and Huang et al. [18], while the CT findings were clearly better than those reported by Zhao et al. [6].

After the 2003 outbreak of SARS, survivors evaluated within three months of discharge showed that lung fibrotic changes occurred mostly in severely sick patients [19]. These same authors also observed that when assessing lung fibrotic changes, DLCO scores were more sensitive than chest radiography and/or high-resolution CT. These results are similar to those observed by our group. During the follow-up of SARS patients, abnormal CT (30%) and impaired DLCO function (15.5%) [20] were still present six months later. These authors also observed significant impairment in DLCO function (23.7%) one year after illness onset [21]. All these data suggest that some of the recovered COVID-19 patients will have significantly impaired lung function months after discharge.

Contrary to our expectations, LDH levels were significantly lower in patients with DLCO abnormalities (<80%). However, serum LDH is a sensitive, burdensome marker for cell injury [22]. One of the reasons could be that its levels vary in multiple circumstances (cell damage related to ischaemia, exposure to bacterial toxins, chemical poisoning, etc.), which is why serum LDH is difficult to use as a valid biomarker of lung damage or inflammation [22]. However, some authors have observed that LDH (cut-off value of 344.5 U/L) could be a predictive factor for early recognition of lung injury and severe COVID-19 cases [23]. These levels are clearly higher than those presented by our patients.

Although the 6 MWT is a validated clinical test designed for use in adults with chronic respiratory disease [24,25], it could be an appropriate test to triage COVID-19 patients. In fact, Huang et al. [18] observed that severe SARS-CoV-2 patients had a greater 6 MWT decline than non-severe patients. In our study, although the distances covered were significantly lower in patients with abnormal DLCO values, these differences disappeared after multivariate analysis.

Sputum induction is a noninvasive method that has been employed to evaluate bronchial inflammation in patients with asthma and other respiratory diseases [26]. This technique allows us to obtain small sputum macrophages that exhibit features of highly active inflammatory cells that could be used to evaluate inflammatory biomarkers [27]. In our study, we considered it necessary to measure both markers in serum and in induced sputum because it was unlikely that analysis of serum markers alone would be enough to reflect an ongoing residual pulmonary inflammatory state. For example, Ropcke et al. [28] observed few and weak correlations between lung and serum markers in chronic obstructive pulmonary disease patients. Contrary to our expectations, we observed that none of the parameters analysed correlated with the alterations observed in pulmonary diffusion.

Multiple mechanisms of lung injury in COVID-19 patients have been described [29]. However, the mechanisms underlying the potential long-term pathogenicity of SARS-CoV-2 have not been studied thus far. For this reason, multiple biomarkers implicated in pulmonary fibrosis were evaluated [30,31]. To the best of our knowledge, they have not been evaluated in COVID-19 patients. Although PAI levels are significantly elevated in the plasma of hospitalised COVID-19 patients [32] and mRNA levels are higher in the lungs of COVID-19 patients than in those of uninfected or influenza patients [33], we did not find differences, perhaps because we analysed a non-acute phase. Finally, ICAM, which is also elevated in the sera of patients with pulmonary fibrosis [34], in the context of the first transcriptomic analysis performed to date on SARS-CoV-2 and IPF, showed that it has a relevant role in both processes [35]. In our study,

we found no significant differences. Thus, we need to continue studying biomarkers that might identify the potential progression of lung damage.

Our study has some limitations. First, this study included a small number of patients, so results should be interpreted with caution. Statistical nonsignificance may not rule out differences between severe and mild/moderate cases. In the same way, there was no comparison group (control group). However, that was not the aim of the study because it was focused on SARS-CoV-2 survivors. Second, at the time we planned this study, there was a deep concern about the potential sequelae of this infection and whether any early intervention was required. Third, although it has been suggested that the viral load of SARS-CoV-2 could be a useful marker for assessing disease severity and prognosis [36], we did not include it by assuming that this was related to the severity of the clinical picture. Fourth, although the use of corticosteroids or other immunosuppressive agents was not evaluated, their use could be supposed based on the severity of the disease. This study was carried out before these agents were considered a therapeutic option for COVID-19 patients. Finally, this study has other strengths such as the decision to include the non-invasive induced sputum procedure.

In summary, this study has shown that a high proportion of severely affected COVID-19 patients show impairment in DLCO measurements in the first few months after symptoms onset. Although its long-term impact is still unknown, DLCO could be considered a useful tool to identify individuals at risk for pulmonary sequelae. It will be necessary to follow these patients for longer periods of time to detect needs and appropriately manage potential lung damage.

Acknowledgments

We thank all the patients and their families involved in this study, as well as numerous doctors, nurses, and people working together to fight against the SARS-CoV-2.

Other collaborators in this project: HU San Pedro (Logroño): L. Perez-Martinez, MA. Martínez, MF. Espada; HCU Lozano Blesa (Zaragoza): P. Crusells, S. Letona, JM. Guzman, L. Anoro, E. García MM. Rivas, A. Prados, J. Izquierdo, R. Pinilla, A. Lainez, R. García, C. Gonzalez, P. Sanchez), HU Reina Sofía de Murcia: C. Alcade, A. Alcaraz, MJ. Alcaraz, A. Cano, A. Muñoz; HU Burgos: MS. Serrano, E. Reguero, E. Arauzo.

Financial support. This work was supported by the “Convocatoria de expresiones de interés para la financiación de proyectos de investigación sobre el SARS-CoV-2 y la enfermedad COVID” from Instituto de Salud Carlos III (Project COV20/00401).

Conflicts of interest. No of the authors reported conflicts of interest.

REFERENCES

[1] Del Rio C, Collins LF, Malani P. Long-term health consequences of COVID-19. JAMA. 2020. doi: 10.1001/jama.2020.19719.

[2] Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-1034.

[3] Zhang Y, Li J, Zhan Y, Wu L, Yu X, Zhang W, et al. Analysis of serum cytokines in patients with severe acute respiratory syndrome. Infect Immun. 2004;72(8):4410-4415.

[4] Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine. 2018;104(2018):8-13.

[5] Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ. 2020;368:m1091. doi: 10.1136/bmj.m1091.

[6] Zhao YM, Shang YM, Song WB, Li QQ, Xie H, Xu QF, Jia JL, Li LM, Mao HL, Zhou XM, Luo H, Gao YF, Xu AG. Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. EClinicalMedicine. 2020;25:100463.

[7] Mo X, Jian W, Su Z, Chen M, Peng H, Peng P, et al. Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. Eur Respir J. 2020;55(6):2001217. doi: 10.1183/13993003.01217-2020.

[8] Wei J, Yang H, Lei P, Fan B, Qiu Y, Zeng B, et al. Analysis of thin-section CT in patients with coronavirus disease (COVID-19) after hospital discharge. J Xray Sci Technol 2020;28(3):383-389.

[9] Frija-Masson J, Debray MP, Gilbert M, Lescure FX, Travert F, Borie R, et al. Functional characteristics of patients with SARS-CoV-2 pneumonia at 30 days post-infection. Eur Respir J. 2020;56(2):2001754.

[10] Report of the WHO-China Joint Mission on Coronavirus Disease. 2019. https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.

[11] Hennus MP, van Vught AJ, Brabander M, Brus F, Jansen NJ, Bont LJ. Mechanical ventilation drives inflammation in severe viral bronchiolitis. PloS One. 2013;8(12):e83035.

[12] ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166(1):111-117.

[13] Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26(5):948-968.

[14] Wegrzynowska-Teodorczyk K, Rudzinska E, Lazorczyk M, Nowakowska K, Banasiak W, Ponikowski P, et al. Distance covered during a six-minute walk test predicts long-term cardiovascular mortality and hospitalisation rates in men with systolic heart failure: an observational study. J Physiother. 2013;59(3):177-187.

[15] Ding X, Xu J, Zhou J, Long Q. Chest CT findings of COVID-19 pneumonia by duration of symptoms. Eur J Radiol. 2020;127:109009.

[16] Djukanovic R, Sterk PJ, Fahy JV, Hargreave FE. Standardised methodology of sputum induction and processing, Eur Respir J Suppl. 2002;37:1s-2s.

[17] Torres-Castro R, Vasconcello-Castillo L, Alsina-Restoy X, Solis-Navarro L, Burgos F, Puppo H, et al. Respiratory function in patients post-infection by COVID-19: a systematic review and meta-analysis. Pulmonology. 2020;25:S2531-0437(20)30245-2.

[18] Huang Y, Tan C, Wu J, Chen M, Wang Z, Luo L, et al. Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. Respir Res. 2020;21(1):163.

[19] Xie L, Liu Y, Xiao Y, Tian Q, Fan B, Zhao H, et al. Follow-up study on pulmonary function and lung radiographic changes in rehabilitating severe acute respiratory syndrome patients after discharge. Chest. 2005;127(6):2119-2124.

[20] Hui DS, Joynt GM, Wong KT, Gomersall CD, Li TS, Antonio G, et al. Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors, Thorax. 2005;60(5):401-409.

[21] Hui DS, Wong KT, Ko FW, Tam LS, Chan DP, Woo J, et al. The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors, Chest. 2005;28(4):2247-2261.

[22] Thomeer M, Grutters JC, Wuyts WA, Willems S, Demedts MG. Clinical use of biomarkers of survival in pulmonary fibrosis. Respir Res. 2010;11(1):89.

[23] Han Y, Zhang H, Mu S, Wei W, Jin C, Tong C, et al. Lactate dehydrogenase, an independent risk factor of severe COVID-19 patients: a retrospective and observational study. Aging (Albany NY). 2020;12(12):11245-11258.

[24] Singh SJ, Puhan MA, Andrianopoulos V, Hernandes NA, Mitchell KE, Hill CJ, et al. An official systematic review of the European Respiratory Society/American Thoracic Society: measurement properties of field walking tests in chronic respiratory disease. Eur Respir J. 2014;44(6):1447-1478.

[25] Holland AE, Spruit MA, Troosters T, Puhan MA, Pepin V, Saey D, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J. 2014;44(6):1428-1446.

[26] Eickmeier O, Huebner M, Herrmann E, Zissler U, Rosewich M, Baer PC, et al. Sputum biomarker profiles in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) and association between pulmonary function. Cytokine. 2010;50(2):152-157.

[27] Frankenberger M, Menzel M, Betz R, Kassner G, Weber N, Kohlhaufl M, et al. Characterization of a population of small macrophages in induced sputum of patients with chronic obstructive pulmonary disease and healthy volunteers, Clin Exp Immunol. 2004;138(3): 507-516.

[28] Ropcke S, Holz O, Lauer G, Muller M, Rittinghausen S, Ernst P, et al. Repeatability of and relationship between potential COPD biomarkers in bronchoalveolar lavage, bronchial biopsies, serum, and induced sputum. PloS One. 2012;7(10):e46207.

[29] Liu J, Zheng X, Tong Q, Li W, Wang B, Sutter K, et al. Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. J Med Virol. 2020;92(5):491-494.

[30] Habibie H, Putri KSSS, Boorsma CE, Brass DM, Heukels P, Wijsenbeek M, et al. Osteoprotegerin is elevated in pulmonary fibrosis and associates with IPF progression. bioRxiv. 2020. doi: https://doi.org/10.1101/2020.12.02.408062.

[31] Pardo A, Selman M, Kaminski N. Approaching the degradome in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol. 2008;40(6-7):1141-1155.

[32] Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol. 2020;7(8):e575-e582.

[33] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med. 2020;383(2):120-128.

[34] Tsoutsou PG, Gourgoulianis KI, Petinaki E, Mpaka M, Efremidou S, Maniatis A, et al. ICAM-1, ICAM-2 and ICAM-3 in the sera of patients with idiopathic pulmonary fibrosis. Inflammation. 2004;28(6):359-364.

[35] Taz TA, Ahmed K, Paul BK, Kawsar M, Aktar N, Mahmud SMH, et al. Network-based identification genetic effect of SARS-CoV-2 infections to Idiopathic pulmonary fibrosis (IPF) patients. Brief Bioinform. 2020. doi: 10.1093/bib/bbaa235.

[36] Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, et al. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis. 2020;20(6):656-657.

Table 1. Patient characteristics among SARS-CoV-2 survivors according to DLCO severity.

	DLCO <80 (n = 52)	**DLCO ≥80 (n = 48)**	***P* value**
Age in years, mean (± SD)	54.98 ±10.72	54.75 ± 9.83	0.911
Age >50 years, n (%)	34 (65.4)	35 (72.9)	0.416
Male sex, n (%)	33 (63.5)	31 (64.6)	0.907
Caucasian, n (%)	47 (90.4)	43 (89.6)	0.894
Never smoker history, n (%)	32 (61.5)	27 (56.3)	0.591
Comorbidities			
Cardiovascular disease, n (%)	4 (7.7)	0 (0)	0.119
Hypertension, n (%)	15 (28.8)	10 (20.8)	0.355
Diabetes mellitus, n (%)	7 (13.5)	3 (6.4)	0.324
Chronic renal failure, n (%)	2 (3.8)	0 (0)	0.496
Chronic aspirin use, n (%)	2 (3.8)	1 (2.1)	1.000
Chronic statin use, n (%)	8 (15.4)	4 (8.3)	0.362
Chronic ACE/ARA-II use, n (%)	9 (17.3)	8 (16.7)	0.932
SARS-CoV-2 data during hospitalization admission			
Severity disease during hospital admission, n (%)	**34 (65.4)**	**13 (27.1)**	**<0.0001**
Days of hospitalization, median (p25, p75)	**7.0 (5.0; 9.75)**	**8.0 (6.0; 11.0)**	**0.038**

Note: ACE = angiotensin converting enzyme inhibitors; ARA-II = angiotensin II receptor blockers; BMI = Body mass index; DLCO = diffusion capacity of the lung for carbon monoxide; SD = Standard deviation

Table 2. Analytical characteristics among SARS-CoV-2 survivors according to DLCO severity.

	DLCO <80 (n = 52)	**DLCO ≥80 (n = 48)**	***P* value**
Serum parameters, median (p25, p75)			
WBC count, cells/μL	6.10 (5.30; 6.59)	5.70 (5.0; 6.6)	0.383
Glucose, mg/dL	**97.0 (93.25; 112.7)**	**32.4 (27.7; 39.9)**	**0.016**
Creatinine, mg/dL	0.87 (0.74; 1.01)	0.87 (0.76; 0.98)	0.970
ALT, UI/L	21.0 (16-0; 32.0)	24.0 (18.0; 33.0)	0.224
AST, UI/L	**22.0 (17.0; 25.0)**	**24.0 (20.0; 27.0)**	**0.034**
LDH, UI/L	**187.0 (164.0; 201.0)**	**196.0 (174.2; 256.7)**	**0.006**
CRP g/dL	3.0 (1.0; 4.0)	4.0 (1.0; 4.0)	0.751
OPG pg/ml	62.6 (48.0; 81.0)	58.0 (48.4; 72.5)	0.410
TIMP-1 ng/ml	278.1 (249.8; 306.8)	281.3 (242.6; 312.3)	0.598
ICAM-1 ng/ml	169.9 (131.5; 245.9)	173.5 (122.5; 243.6)	0.738
ICAM-3 ng/ml	141.8 (115.8; 187.9)	136.6 (106-2; 163.0)	0.143
PAI-1 ng/ml	125.6 (98.2; 146.5)	119.2 (107.3; 142.4)	0.945
PAI-2 ng/ml	4.3 ±(2.8; 6.7)	3.7 (2.1; 5.2)	0.492
Induced sputum samples, median (p25, p75)			
OPG pg/ml	1.0 (1.0; 9.71)	1.0 (1.0; 11.1)	0.912
TIMP-1 ng/ml	68.9 (35.0; 120.4)	48.5 (35.0; 75.1)	0.112
ICAM-1 ng/ml	2.2 (0.9; 3.5)	2.21 (0.1; 2.38)	0.083
ICAM-3 ng/ml	34.2 (12.6; 86.1)	38.27 (16.2; 92.4)	0.812

Note: ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; CRP = C-reactive protein; DLCO = diffusion capacity of the lung for carbon monoxide; ICAM = Intracellular adhesion molecule; LDH = Lactate dehydrogenase; OPG = Osteoprotegerin; PAI = Plasminogen activator inhibitor; TIMP = Tissue inhibitor of matrix metalloproteinase; WBC = White blood cell count.

Table 3. Pulmonary function test and computed tomography among SARS-CoV-2 survivors according to DLCO severity.

	DLCO <80 (n = 52)	**DLCO ≥80 (n = 48)**	***P* value**
Days after symptoms onset*	**100.0 (87.5; 108.7)**	**114.5 (94.2; 133.7)**	**0.012**
Days after symptoms onset >90, n (%)	36 (69.2)	38 (79.2)	0.258
Functional lung parameter and imaging CT			
FVC (%)*	106.9 (91.0; 113.7)	104.5 (94.7; 114.7)	0.904
FVC >80%, n(%)	48 (92.3%)	46 (95.8%)	0.906
FEV1 (%)*	102.5 (94.1; 113.0)	107.2 (98.0; 118.0)	0.214
FEV1 >80%, n (%)	48 (91.7)	44 (91.7)	0.458
FEV1/FVC ratio*	1.0 (0.9; 1.0)	0.97 (0.92; 1.01)	0.066
6MWT distance, mean (± SD)	**513.0 (450.0; 594.6)**	**577.0 (540.0; 645.0)**	**0.001**
6MWT distance >550,n (%)	**20 (39.2)**	**31 (66)**	**0.008**
Pathologic CT, n (%)	31 (59.6)	20 (42.6)	0.900

Note: Data presented as median (P25; P75)*; 6MWT = 6-minute walk test; CT = chest-computed tomography; DLCO = diffusion capacity of the lung for carbon monoxide; FEV1 = forced expiratory volume in the first second; FVC = forced vital capacity; SD = Standard deviation